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Imposex level and penis malformation in *Hexaplex trunculus* from the Tunisian coast

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Abstract: *Hexaplex trunculus* (Linnaeus, 1758) is a gonochoric marine gastropod. Previous studies demonstrated that the biocide TBT (tributyltin) induced a sexual abnormality known as imposex (superimposition of male sexual characters onto females) in this whelk. Our study showed imposex in 19 stations out of 20 along the Tunisian coast. The frequency of imposex ranged from 0 to 100%. Among the 19 sites where the condition was found, 8 were considered as highly affected by imposex (VDSI > 3.7), 6 were moderately affected (VDSI > 1.3), and 4 were slightly affected (VDSI > 0). The most affected population was observed in the Bizerta Channel where the highest boating traffic was recorded; no imposex features were found in the Sea of Zarat where boating traffic was very low. Significant differences in imposex levels were obtained among sites with low, moderate, and high boating traffic. All the imposex indices values (I%, RPSI, RPLI, VDSI, FPL, and VDL) were significantly more elevated at sites with high boating traffic compared with sites with low and moderate boating traffic. Malformations of the penis were observed only in five stations and in very low rates, but where imposex rates were high. The incidence of penis malformation in males was significantly related to the boating traffic, I%, and VDSI. However, in females, a correlation was obtained only for the RPLI. The present study provides data on imposex level and penis malformations in *H. trunculus* from the Tunisian Coast that could be used as a starting point for future monitoring programs and for temporal trend surveillance related to TBT pollution in Tunisia where the use of TBT is not yet banned.

Key words: Muricidae, TBT-biomarker, marine pollution, Tunisia

Imposex (Smith 1971) or pseudo-hermaphroditism (Jenner 1979) is the development of male sexual characteristics (*i.e.*, a penis and/or a sperm-duct) in female prosobranch gastropods. The active biocide used in anti-fouling paints, TBT, was suspected to be the major cause of imposex since a direct correlation with shipping intensities was established (Bryan *et al.* 1986, Gibbs and Bryan 1986, Ten Hallers-Tjabbes *et al.* 1994, Axiak *et al.* 1995, Harino *et al.* 1998, Rilov *et al.* 2000, Fernandez *et al.* 2002). In contrast, even though there is little doubt that TBT has been the main cause of imposex in gastropods, it may be not the sole cause. Nias *et al.* (1993) reported that exposure to copper induces imposex. The same condition was also noted by Evans *et al.* (2000) in *Nucella lapillus* (Linnaeus, 1758) exposed to the estrogen-mimic nonylphenol. However, Davies *et al.* (1987) found imposex in 12% of the *N. lapillus* from a non-polluted site and considered it a natural phenomenon. Imposex has now been observed in about 150 gastropod species (Oehlmann *et al.* 2000).

In *Hexaplex trunculus,* observations on imposex were first reported along European coasts by Martoja and Bouquegneau (1988) in France, Axiak *et al.* (1995) in Malta, Terlizzi *et al.* (1998) in Italy, Vasconcelos *et al.* (2006) in Portugal, Rilov *et al.* (2000) in Israel, and Lahbib *et al.* (2004) in Tunisia. Some authors have indicated the development of malformations of the penis in males as well as in females of some gastropod species, namely *Hinia reticulata* (Linnaeus, 1758) (Stroben *et al.* 1992), *H. trunculus* (Terlizzi *et al.* 1998, Vasconcelos *et al.* 2006), and *Bolinus brandaris* (Linnaeus, 1758) (Ramon *et al.* 2001). However, descriptions of the penis malformations in *H. trunculus* were limited. The aim of the present work was (1) to provide data on imposex levels along the Tunisian Coast and (2) to describe malformations of the penis in *H. trunculus.*

MATERIALS AND METHODS

Adult *Hexaplex trunculus*, with shells of 40 to 60 mm in height and a sample size of 44 to 150 specimens per station, were collected between March and July 2004 from Tunisian coastal waters. Twenty locations were chosen according to the intensity of the marine traffic, from Bizerta to Djerba (Table 1, Fig. 1). For each zone, both the type of boating activity and the boat density were recorded. The number of working fishing boats in each area was obtained from annual statistical data of fishing activity in Tunisia in 2004 provided by the Ministry of Agriculture, or directly in some sites from fishermen. In sites with commercial activity, the number and type of boats were obtained from the annual report of the Tunisian commercial marine and harbors office in 2003.

Table 1. Collection data at the various stations. BT, boat type (F, fishing boat; P, passenger liner; M, merchant ship; O, oil tanker; F_b, ferry-boat); FD, fishing-boat density expressed in number of working boats in the area in 2004; CD, commercial boat traffic expressed in number of boats to/from the area in 2003; TC, traffic category (H, high; Md, moderate; L, low; *, site with low boating density but located nearby a commercial traffic line or harbor); M, male; F, female; *N*, number of individuals; SL, average shell length (mm); I%, imposex frequency; PL, average penis length (mm); RPSI, relative penis size index; RPLI, relative penis length index; VDSI, vas deferens sequence index; VDSr, vas deferens sequence range; VDL, mean vas deferens length (mm).

Site	BT	FD	CD	TC	Sex	*N*	SL	I %	PL	RPSI	RPLI	VDSI	VDSr	VDL
1. Bizerta Channel	FPMO	505	1014	H	M	63	41.7		12.05					
					F	37	46.5	100	8.23	33.03	69.13	4.24	4-5	12.17
2. Quarries Bay	F	120	—	H	M	26	41.9		11.81					
					F	37	49.2	100	7.77	28.47	65.79	4.09	4-4.3	12.29
3. Menzel Abderrahmen	F	150	—	H	M	33	44.9		14.64					
					F	29	41.7	100	5.18	5.13	37.16	3.73	3-4.7	12.12
4. Menzel Bourguiba	F	30	SA	H	M	29	52.9		12.25					
					F	27	55.7	100	3.54	2.69	29.96	3.97	3-4.7	12.20
5. Menzel Jemil	F	60	—	Md	M	20	44.1		13.56					
					F	40	43.1	66.6	0.28	0.00	2.05	1.31	0-4.3	3.53
6. El Azib	F	50	—	Md	M	29	43.9		14.33					
					F	21	46.2	62.0	0.37	0.00	2.52	1.28	0-3	2.64
7. Tunis North Lake	FPM	78	2444	H*	M	14	48.6		17.41					
					F	30	49	100	3.60	0.88	20.68	3.97	2-4.3	12.90
8. Small Gulf of Tunis	FPM	0	3001	H*	M	48	55.8		14.32					
					F	42	56.6	85.7	1.38	0.08	9.63	3.27	0-4.7	11.69
9. Khniss Lagoon	F	64	—	L	M	43	45.9		17.49					
					F	25	47.1	40	0.17	0.00	0.01	0.75	0-2	2.03
10. NPK Sfax	M	—	SA	H	M	51	42		10.31					
					F	30	44.1	100	5.63	16.98	55.38	4.00	4-4.3	12.91
11. Sfax Fishing Harbor	F	736	—	H	M	14	51.1		24.72					
					F	32	52.3	100	8.16	3.62	33.08	4.21	4-4.7	13.19
12. Gargour	F	30	—	H*	M	65	44.8		10.29					
					F	72	43.1	93.0	0.83	0.05	8.00	3.32	0-4	11.24
13. Skhira	FO	38	236	H	M	38	48.5		15.36					
					F	32	50.2	84.4	1.06	0.03	6.96	2.53	0-4	8.26
14. Gabes Fishing Harbor	F	108	—	H	M	46	52.5		14.78					
					F	33	54.2	51.5	1.60	0.02	5.48	1.82	0-4	5.91
15. Sea of Zarat	F	10	—	L	M	70	52.1		13.86					
					F	80	54.9	0.0	0.00	0.00	0.00	0.00	0	0.00
16. Adjim Channel	F_b	244	4	H	M	18	50.2		18.20					
					F	37	53.9	100	5.80	2.97	31.02	4.09	3-4.7	13.13
17. Gigthis-Djorf	F	20	—	L	M	62	42.9		9.16					
					F	81	46.6	6.2	0.02	0.00	0.09	0.13	0-3	1.70
18. Gigthis	F	52	—	Md	M	61	42.8		13.14					
					F	88	43	19.7	0.07	0.00	0.29	0.60	0-4	2.05
19. Guallala	F	10	—	L	M	26	42.9		14.28					
					F	27	46.6	3.7	0.00	0.00	0.05	0.04	0-1	0.10
20. Ain Meider	F	8	—	L	M	37	42.5		9.53					
					F	28	42.9	3.6	0.00	0.00	0.00	0.04	0-1	0.11

In the laboratory, the 1621 collected individuals were frozen. The shell was broken after thawing, and the soft tissues carefully removed. The mantles were longitudinally cut to reveal the pallial oviduct in females. Sexes were determined according to the presence or absence of the capsule gland and vagina. Normal females were separated from abnormal ones using a binocular dissecting microscope. In males and imposex-affected females, the length of the

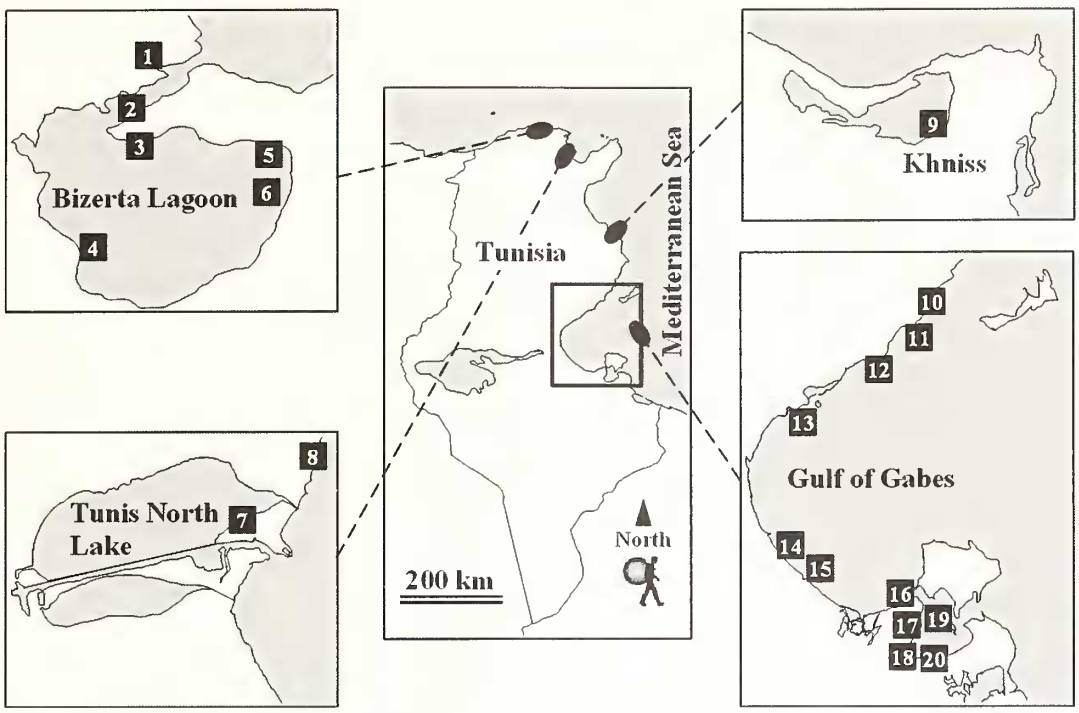

Figure 1. Sampling sites of *Hexaplex trunculus* along the Tunisian coast. 1, Bizerta Channel; 2, Quarries Bay; 3, Menzel Abderrahmen; 4, Menzel Bourguiba; 5, Menzel Jemil; 6, El Azib; 7, Tunis North Lake; 8, small gulf of Tunis; 9, lagoon of Khniss; 10, NPK Sfax; 11, fishing harbor of Sfax; 12, Gargour; 13, Skhira; 14, fishing harbor of Gabes; 15, Sea of Zarat; 16, Adjim Channel; 17, Djorf-Ghigthis; 18, Ghigthis; 19, Guallala; 20, Ain Meidder.

straightened penis (from the base of the penis to the end of the penial flagellum) and the vas deferens were measured using an ocular micrometer.

Imposex incidence and levels were quantified by using the following indices: (1) the imposex incidence or frequency (I% = percentage of imposex-affected females compared to the total number of females in the sample), (2) the relative penis size index [RPSI = (average length of female penises)^3 × 100/(average length of male penises)^3] according to Bryan *et al.* (1986), (3) the relative penis length index [RPLI = (average length of female penises × 100)/(average length of male penises)] according to Stewart *et al.* (1992), (4) the female penis average length (FPL), (5) the female vas deferens average length (VDL), and (6) the vas deferens sequence index [VDSI = (sum of imposex stage values of all females)/(total number of females)] following Gibbs *et al.* (1987). Some imposex stages and malformations affecting the penis were photographed under the binocular microscope using a digital camera.

For the statistical comparison of the data, each sampling station was assigned to 1 of 3 categories in terms of boating traffic density (Table 1): (1) high shipping density (>100 boats in the area per year), (2) moderate (50-100 boats in the area per year), and (3) low (<50 boats in the area per year). Sites with shipyard activities or located near a high traffic area were classified in the high category. All imposex indices (I%, RPSI, RPLI, VDSI, FPL, and VDL) were calculated for each category. The significance of differences in imposex levels between the 3 different categories of boating traffic were tested using one-way analysis of variance (ANOVA) and Chi-square test.

With regard to the malformation affecting the penis, the relationship with boating traffic and some imposex indices (I%, RPLI, and VDSI), recorded at sites where the condition was observed, was established, using a regression analysis.

RESULTS

Imposex distribution

Imposex was observed at 19 out of 20 sampling sites. However, the degree and the intensity of this alteration varied depending on the boating activity at each site (Table 1). The results indicated that the Bizerta Channel had the highest recorded imposex level with an RPLI of 69.08%, a VDSI of 4.23 and a sterility rate of 8%. High levels of imposex were also observed for Quarries Bay, Menzel Abderrahmen, Men-

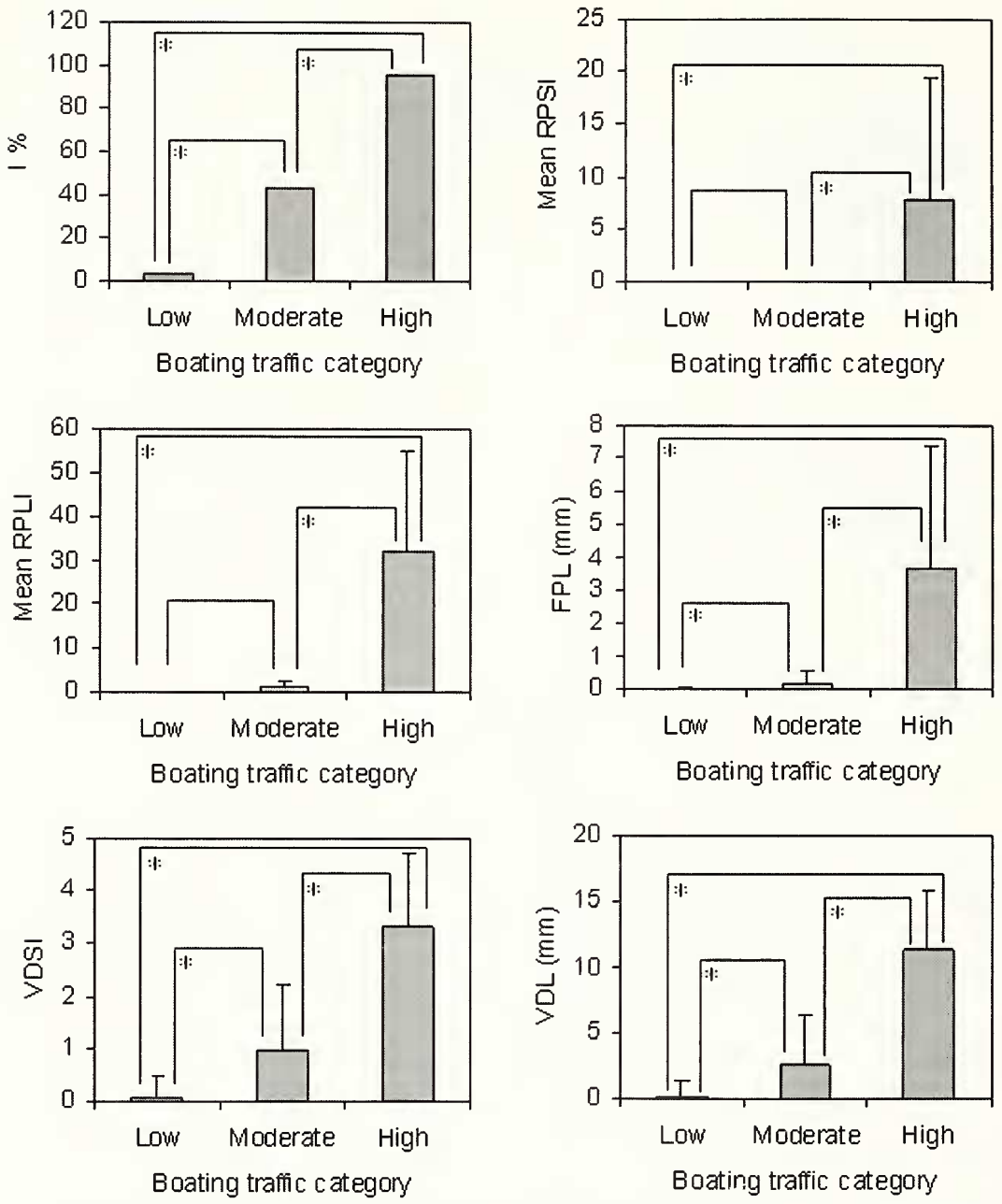

Figure 2. Imposex development in *Hexaplex trunculus* in relation to boating traffic. (*), significant difference ($P = 0.05$, one-way ANOVA applied to all indices except the I% index tested by a Chi-square test χ^2).

zel Bourguiba, Tunis North Lake, NPK Sfax, Fishing Harbor of Sfax, and Adjim Channel (VDSI above 3.7, Table 1). Moderate levels of imposex were recorded for Gargour, Skhira, fishing harbor of Gabes, Khniss, Small Gulf of Tunis, El Azib, and Menzel Jemil (VDSI >1.3, Table 1). At the rest of the stations (Guallala, Ain Meider, Gigthis-Djorf, and Gigthis), very low levels of imposex were recorded (VDSI above 0), while no female showing any form of genital disorder was observed at the Sea of Zarat (Table 1).

Significant differences were obtained between imposex indices and categories of boating traffic, indicating that imposex development is related to the intensity of marine traffic (Fig. 2). All the imposex indices values were significantly elevated in the high shipping traffic category of stations

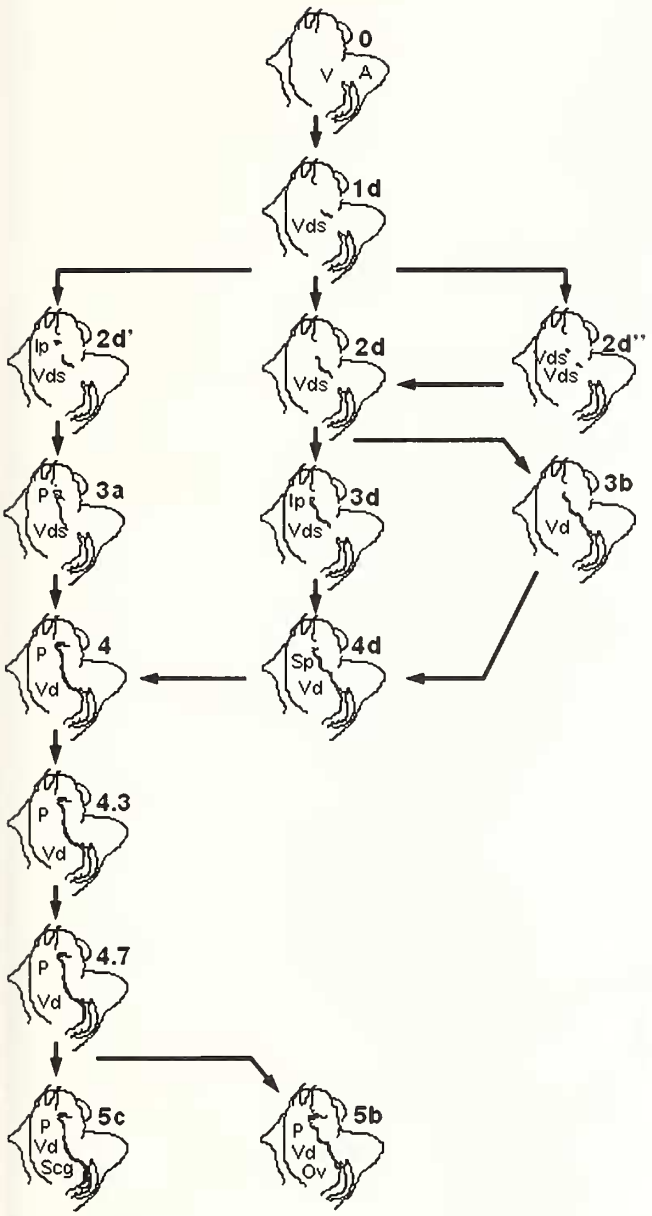

Figure 3. Schematic representation of the imposex pathways observed in *Hexaplex trunculus* from Tunisian waters. A, anus; Scg, split capsule gland; Ip, incipient penis; Ov, occluded vulva; p, penis; sp, small penis; V, vulva; Vd, vas deferens; Vds, vas deferens section.

compared to the moderate and low categories. Significant differences were also revealed between the moderate and the low categories for all imposex indices except RPSI and RPLI (Fig. 2).

Scheme of imposex pathways

The sequential stages of the imposex development observed in this study are illustrated (Fig. 3). The first imposex characters (1d, 2d, 2d', and 2d'') were detected only at sites with low boating activity. Whereas some advanced stages (3b, 3d, 4d, 4, 4.3, and 4.7) were found at the more affected areas (Fig. 3). The more advanced stages of imposex recorded in this study were the VDS 5b and 5c. These stages, causing sterility in females, were only revealed in the Bizerta Channel.

In *Hexaplex trunculus*, females start to show evidence of imposex by the presence of a vas deferens section halfway between the penis site and the vagina (stage 1d). Thereafter, 3 cases are possible: (1) the vas deferens grows towards the proximal and distal sides (stage 2d), (2) an incipient penis develops behind the right ocular tentacle (stage 2d'), or (3) a second portion of the vas deferens appears (stage 2d''). At stage 3, females could show (1) a complete vas deferens without a penis (stage 3b), (2) an incipient penis linked to the vas deferens section (stage 3d), or (3) a small penis with penis-duct continuing in a portion of the vas deferens (stage 3a). At stage 4, the vas deferens reaches the vaginal opening, passes it (stage 4.3), and runs into the ventral portion of the capsule gland (stage 4.7). Sterility is reached in stage 5, in which the vulva is occluded by the development of the vas deferens tissue at the spawning aperture (stage 5b, Fig. 4A-B) or the capsule gland is split following the ventral development of the vas deferens (stage 5c, Fig. 4C).

Penis malformations in males

The normal male penis in *Hexaplex trunculus* is generally bent (Fig. 5A), it possesses a large base and a long fla-

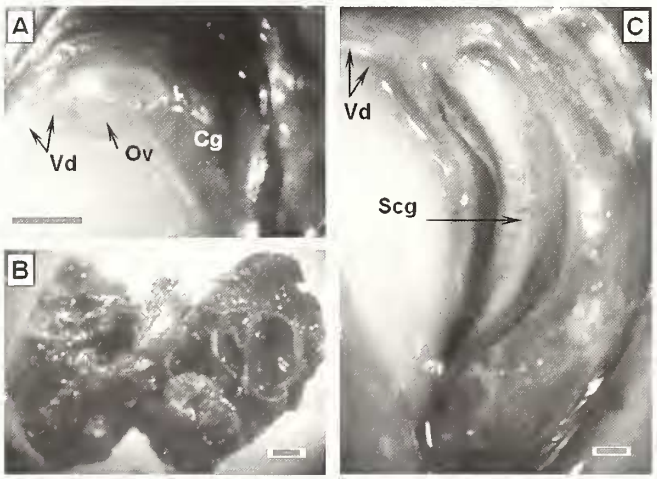

Figure 4. Imposex stage 5b (A, occluded vulva; B, dark mass of egg capsules removed from the capsule gland at this stage) and 5c (C) showing the split capsule gland. Cg, capsule gland; Scg, split capsule gland; Ov, occluded vulva; Vd, vas deferens (scale bar = 1 mm).

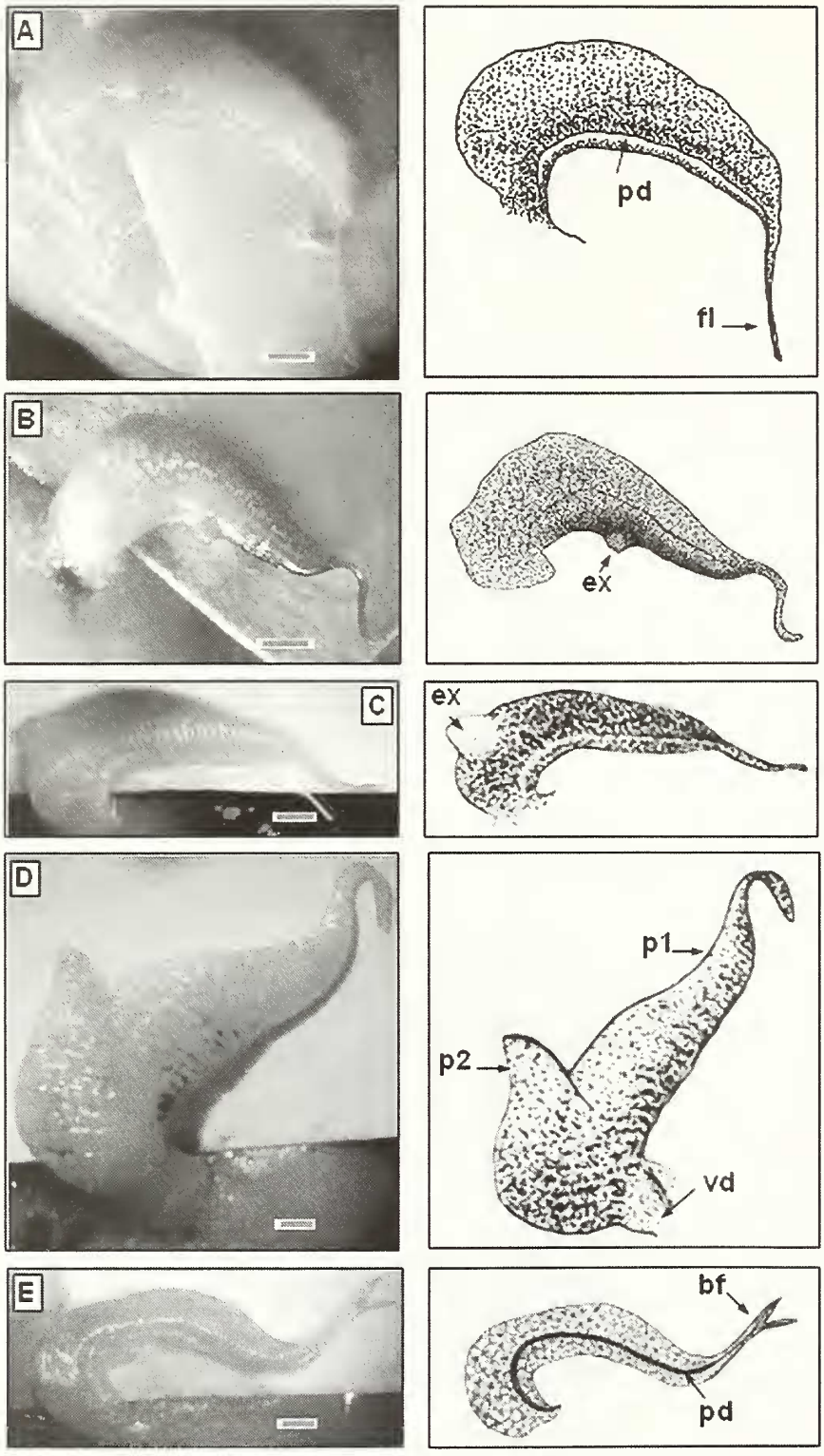

Figure 5. Penis malformations in male *Hexaplex trunculus*. A, normal penis. B-E, abnormal penises observed in Bizerta Channel (B), the small Gulf of Tunis (C-D) and the Tunis North Lake (E). bf, bifurcation; pd, penis duct; ex, excrescence; fl, flagellum; p1, penis 1; p2, penis 2; vd, vas deferens (scale bar = 1mm).

gellum-like tip reaching on fifth of the total penis length, depending on the reproductive season. The penis-duct is an open fissure, but it becomes a closed tube during reproductive activity. Male penis malformations were detected in the Bizerta Channel, Tunis North Lake, and the small gulf of Tunis with 1-2 affected individuals per station (Table 2). The malformation varied from the development of a tissue bud in both the anterior and the posterior sides of the penis (Fig. 5B-C) to the biphallic penis (Fig. 5D) and the bifurcated flagellum (Fig. 5E). A good correlation was obtained between the incidence of penis malformation, boating traffic, I%, and VDSI ($r > 0.85$, Fig. 6). However, the correlation with RPLI was moderate ($r = 0.49$).

Penis malformations in female

Malformations affecting the female penis were observed in 4 stations with a number varying from 1 to 3 affected individuals per station (Table 2). In Gabes and Bizerta Channel, malformations were characterized by the development of the bud tissue (excrescence) at the base of the penis (Fig. 7A) or half of the total penis length at the posterior side (near the penis-duct, Fig. 7B). Biphallic penis (Fig. 7C) and inflated penis tip (Fig. 7D) were revealed in Menzel Jemil and Tunis North Lake, respectively. The regression analysis showed a good relationship of the malformation rate only with RPLI. The correlations between boating traffic and I% and VDSI were relatively weak ($r < 0.34$, Fig. 6).

DISCUSSION

Imposex was found in most of the sites. High levels were recorded in sites frequently used by boats or located near a source of leaching TBT, such as harbors and shipyard activities stations. Moreover, the highest indices were recorded in areas where the predominant source of TBT involved large boats. However, the moderate level of imposex recorded in the fishing harbor of Gabes

Table 2. Genital malformation in *Hexaplex trunculus* from Tunisian coast with comparison to data collected from previous studies in the same snail and other species of gastropod. *N*, number of specimens; Na, number of affected individuals; VDS, vas deferens sequence; —, no data available.

			Males			Females			
Studies	Species	Locations	*N*	Malformation	Na	*N*	Malformation	Na	VDS
Present study	*Hexaplex trunculus*	Gabès Fishing Harbor	46	Absent	0	33	Penis excrescence	1	4
	Hexaplex trunculus	Steg Tunis North Lake	14	Bifurcated tip	1	30	Inflated penis tip	1	3
	Hexaplex trunculus	Small Gulf of Tunis	48	Double penis	1	42	Absent	0	—
				Penis excrescence	1				
	Hexaplex trunculus	Menzel Jemil	40	Absent	0	40	Double penis	1	2
	Hexaplex trunculus	Bizerte Channel	42	Penis excrescence	2	52	Penis excrescence	3	4.3, 4.7, and 5
Terlizzi *et al.* 1998	*Hexaplex trunculus*	Italian Coast	—	Bifurcated penis	—	—	Bifurcated penis	—	>4
				Branched vas deferens			Branched vas deferens	—	>4
Vasconcelos *et al.* 2006	*Hexaplex trunculus*	Ria Formosa (Portugal Coast)	621	Penis excrescence	2	562	Rounded penis tip	5	—
Ramon *et al.* 2001	*Bolinus brandaris*	Villanovai (Spain Coast)	59	Penis excrescence	1	75	Absent	0	—
Stroben *et al.* 1992	*Hinia reticulata*	Brittany and Normandy Coast	2760	Penis and/or vas deferens excrescences	4	3562	Bifurcated penis and double penis	10	4
							Coiled vas deferens	7	—

(51.5%), despite the high boating traffic recorded in the area (108 boats), is explained by the heterogeneity of the sampled population. At this station, many fishermen reject accidentally fished whelks found in fishing nets that come from locations far from the harbor where no imposex is present. According to imposex index values and the knowledge that TBT is the cause factor of imposex induction (Ten Hallers-Tjabbes *et al.* 1994, Mensink *et al.* 2002), the Bizerta Channel is considered as the most affected site along the Tunisian coast by TBT pollution, because sterility was recorded. The absence of imposex in the Sea of Zarat is explained by the weak intensity of boating traffic recorded at this site. The imposex level in *Hexaplex trunculus* in the present study, in comparison to other Mediterranean countries such as Italy (Terlizzi *et al.* 1998) and Malta (Axiak *et al.* 1995), suggests the Tunisian coast is relatively less polluted by TBT. Sterility was recorded in only one station among 20 with a frequency of 8%. Along the Italian coast, Terlizzi *et al.* (1998) reported the occurrence of sterility in 12 sites out of 15 with a frequency varying from 11.1% at Forio to 100% at SM di Pagana. In Malta, the most affected site had an RPSI of 98.1% and a VDSI of 4.8 (Axiak *et al.* 1995), compared to 33.03 and 4.23 in Tunisia. In the lagoon of Venice (Italy), Pellizatto *et al.* (2004) recorded a female penis length (FPL) of 12 mm, a RPSI of 36.02, and a VDSI of 4.9 in S. Nicolo del Lido, and 8, 8.03 and 4.1 in S. Maria del Mare. Compared with our study, FPL and VDSI indicated that imposex level is relatively similar in Quarries Bay (FPL = 7.77, VDSI = 4.09) and S. Maria del Mare. However, the difference observed in RPSI values (28.47 in Quarries Bay and 8.03 in S. Maria del Mare) is certainly related to the reproductive season in which males exhibit penis length variation. For this reason, we think that using the FPL to asses TBT pollution is more informative than using RPSI. According to Pellizatto *et al.* (2004), the level of TBT found in the entire organism of *H. trunculus* from S. Maria del Mare varied between 53 and 60 ng per g dry weight.

The morphological expression of imposex in Tunisian populations of *Hexaplex trunculus* was different at early stages from the general scheme of vas deferens sequence proposed for the same species in the Maltese Islands (Axiak *et al.* 1995) and Italian coast (Terlizzi *et al.* 1999). These authors mentioned that the first sign of imposex is the presence of an incipient penis behind the right ocular tentacle (stage 1a). Afterwards, the penis duct appeared (stage 2a) and the vas deferens developed progressively from the base of the penis toward the vaginal opening (stage 3a). In Tunisian waters, we observed that the first sign of imposex was expressed by development of a small portion of vas deferens halfway between the penis site and the vagina (stage 1d). Thereafter, the penis appeared following the d pathway (at stage 3d) or the d' pathway (at stage 2d'). These differences in imposex developmental stages observed between Tunisia and Italy and Malta could be explained by factors other than TBT. These factors could range from exposure to heavy metals (Nias *et al.* 1993), parasites (Gorbushin 1997), and other androgenic compounds (Cajaraville *et al.* 2000). Another hypothesis is genetic differences between Tunisian and European populations of *H. trunculus* that could also lead to distinct sequences of imposex induction. The development of a portion of the vas deferens as a first sign of imposex in Tunisian *H. trunculus* allowed calculating a new index, the VDL (mean length of the vas deferens), that gives more information on the level of imposex in the less affected area,

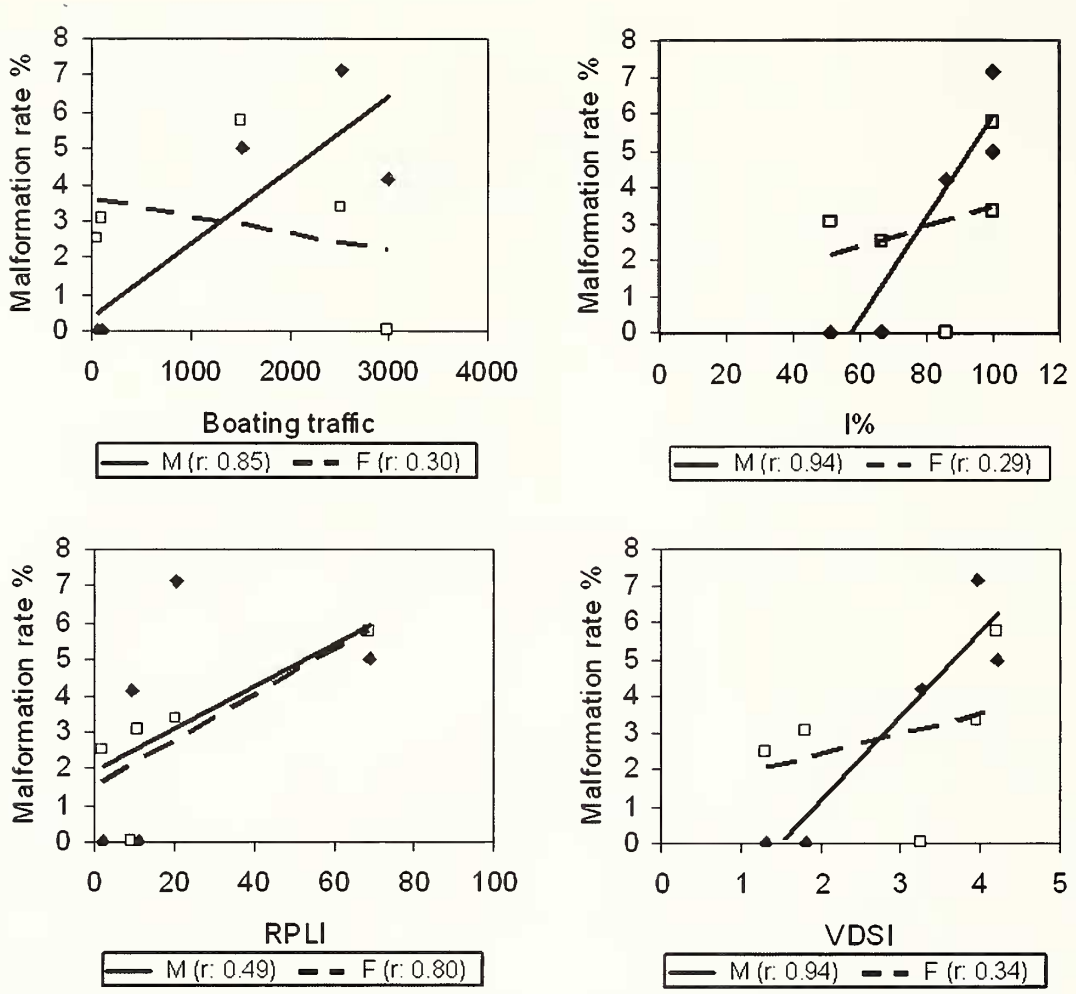

Figure 6. Regression analysis between penis malformation rate and boating traffic and some imposex indices (I%, RPLI, and VDSI) in both sexes. M, in males; F, in females; r, regression coefficient. Boating traffic was estimated as the number of existing boats and visiting boats in the area per year.

where penises in females are lacking or less developed, and RPSI and RPLI are close to 0. These indexes (RPSI and RPLI) are more informative in populations exhibiting the imposex (a) pathway (penis developed at first).

Penis morphological alterations in *Hexaplex trunculus* affected by imposex were reported by earlier authors, but no description nor pictures and schemes were provided. Terlizzi *et al.* (1998) observed penis bifurcation in *H. trunculus* in both sexes, but especially in males from the Italian coast. In the Ria Formosa lagoon (Portugal), Vasconcelos *et al.* (2006) found 2 males among 621 examined with penis excrescences and 5 females among 562 with a rounded penis tip. Compared to our study, the novelty was the observation for the first time of a penis with bifurcated flagellum in males and a penis with excrescence in females. No more than one excrescence per penis was observed in the present study, l in males and females, in comparison to male *Bolinus brandaris*, in which penises with many excrescences were found (Ramon *et al.* 2001). In male *Hinia reticulata* (Stroben *et al.* 1992) and in both sexes of *Nucella lapillus* and *Ocenebra erinacea* (Linnaeus, 1758) (Oehlmann *et al.* 1991, 1992), penis excrescences were also observed (Table 2). Stroben *et al.* (1992) have found females with bifurcated penises and females with two penises in *Hinia reticulata*. Contrary to the present study where no malformations associated to vas deferens development were revealed, Terlizzi *et al.* (1998) found branched vas deferens in *H. trunculus*, and Stroben *et al.* (1992) observed coiled vas deferens in *Hinia reticulata*.

With regard to the imposex stages in females in which the malformations were found, the abnormalities were observed since stage 2d', but especially in the more advanced stages. The correlation between malformed penises and the

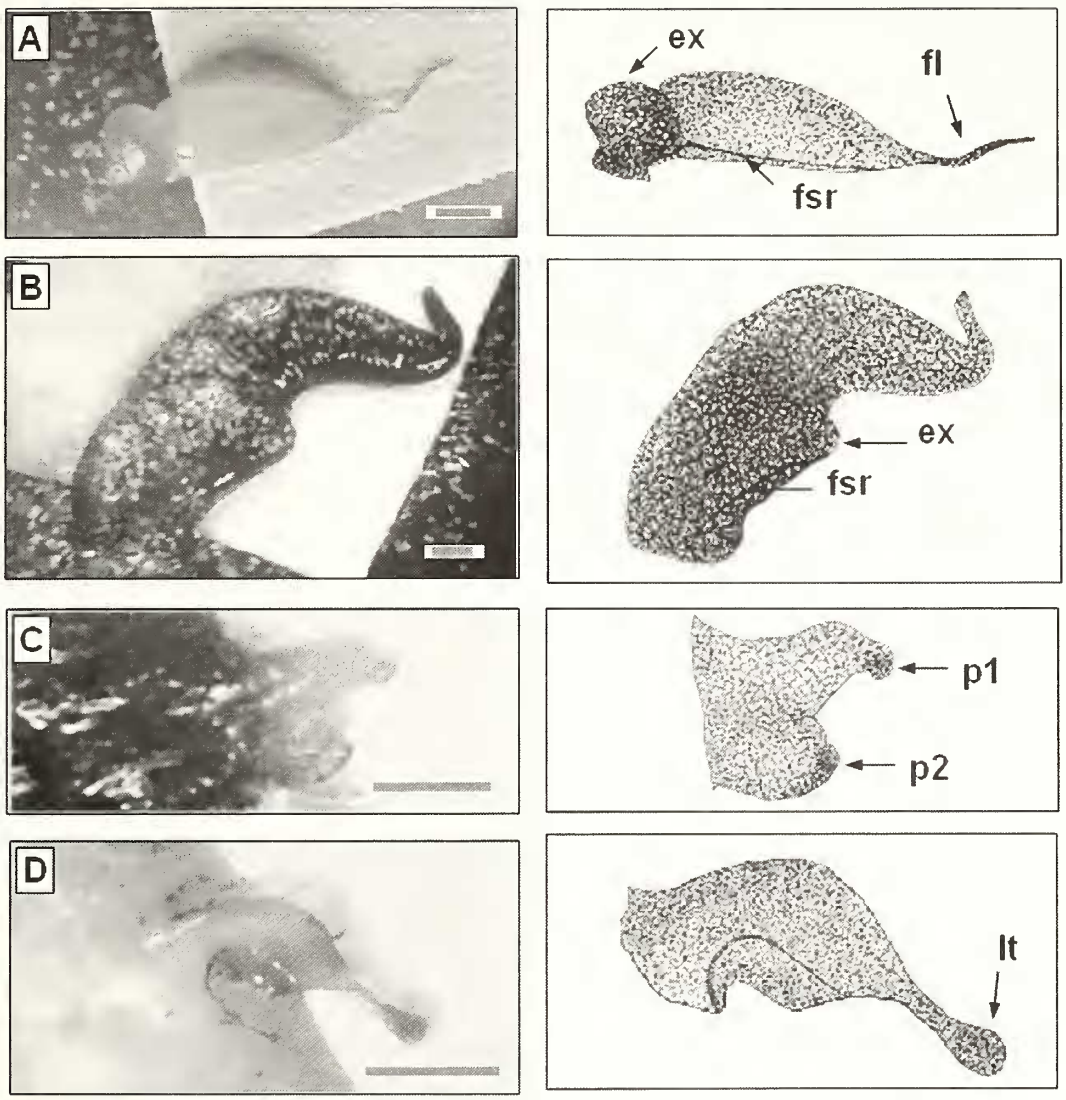

Figure 7. Penis malformations in female *Hexaplex trunculus*. A, in Gabes fishing harbor; B, in Bizerta Channel; C, in Menzel Jemil; D, in Tunis North lake; ex, excrescence; fl, flagellum; fsr, fissure of the vas deferens; lt, inflated tip; p1, penis 1; p2, penis 2 (scale bar = 1 mm).

boating traffic and imposex indices was significantly better in males than in females, except for the RPLI where the converse situation was recorded. These findings indicated that in males, the development of penis malformation is related to imposex level. However, in females, the occurrence of such conditions is related to the development of the penis and consequently to the imposex pathway (d or d'). The causal factor of penis malformation during development is unknown; it could be related to the presence of TBT in sea water, as reported by Mensink *et al.* (2002). These authors obtained in the laboratory penis malformations in juveniles of *Buccinum undatum* at 500 ng/L TBT. In our case, the development of such a condition in males is certainly linked to TBT pollution, because a good correlation with I% and VDSI was obtained. However, this hypothesis must still be supported by laboratory exposure of *H. trunculus* to TBT. Another hypothesis relates to the natural development of malformations, since the rate was low and relatively similar between males and imposex females. In this context, Garaventa *et al.* (2006) have reported the presence of biphallic males among museum specimens of *H. trunculus* collected before the use of TBT. Such data suggest the existence of natural abnormalities of the penis that are not related to TBT pollution.

This paper, reporting the imposex level and penis malformations in *Hexaplex trunculus* from the Tunisian coast, is important to assess the environmental consequences of the boating activity. The high values of VDSI in some populations suggest the existence of diffuse TBT pollution along the majority of the Tunisian coast. Further investigation of organotin in seawater, sediments, and gastropod tissue are in progress. Such data could be useful in regulation of TBT-based antifoulants and the legislation to ban the use of TBT-based paints in Tunisia.

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